

April 12, 2004



EXTRA! EXTRA! READ ALL ABOUT IT!

TOP STORIES:

**** “NEW!!!”** denotes papers that are new to the manuscript update or have switched categories (ex. Submitted to In Press)

**** Remember!** Entire manuscripts can be found in the biotoxins folder in “users on Spottail” (in the same folder as workshop presentations).



SUBMITTED:

**** NEW!!! RESPONSES OF STRESS PROTEINS, ANTIOXIDANT ENZYMES, AND PHOTOSYNTHETIC EFFICIENCY TO HEAT AND OXIDATIVE STRESS IN THE FLORIDA RED TIDE DINOFLAGELLATE, *Karenia brevis* (CL# 1137, Journal of Phycology)** ... Jeanine Miller and Fran VanDolah

ABSTRACT

The environmental conditions under which *Karenia brevis* Hansen and Moestrup often exists are generally considered non-ideal for dinoflagellate growth. The cellular mechanisms by which *K. brevis* adapts to adverse or changing environmental conditions are largely unknown. Thus, in this study we sought to identify stress proteins and antioxidant enzymes that may play a role in the adaptive mechanisms of *K. brevis*. Heat shock protein 60 (Hsp 60), mitochondrial small heat shock protein (mitosHsp), chloroplastic small heat shock protein (chlshHsp), Mn superoxide dismutase (SOD), and Fe SOD were identified by western blotting and their induction in response to heat and oxidative stress was characterized. In addition, changes in F_v/F_m , a measurement of photosynthetic efficiency commonly used as a proxy of cellular stress, were determined in parallel to the responses of these proteins. The response of laboratory cultures to four different stressors was assessed: elevated temperatures, hydrogen peroxide, lead chloride, or elevated light intensities. Hsp 60, Fe SOD, and Mn SOD, expressed under control conditions, were further induced following heat or oxidative stress. MitosHsp, absent under control conditions, responded only to heat stress. In contrast, chlshHsp, absent from controls, responded only to H_2O_2 -induced oxidative stress. The expression of stress proteins and antioxidant enzymes appears to be a more sensitive indicator of heat or chemically-

induced stresses than F_V/F_M ; whereas F_V/F_M decreased significantly in response to elevated light intensities that did not induce the expression of stress proteins. These results identify for the first time several stress proteins and antioxidant enzymes present and responsive to cellular stress in *K. brevis* and provide evidence for differential sensitivity of different cellular compartments to heat versus oxidative stress.

**** NEW!!! QUANTITATIVE CIGUATOXIN ANALYSIS IN FORTY GREAT BARRACUDA (*Sphyraena barracuda*) COLLECTED FROM THE FLORIDA KEYS, USA: COMPARISON OF TWO DETECTION METHODS (CL# 1132, Environmental Health Perspectives)** ... Marie-Yasmine Bottein Dechraoui, Jessica Tiedeken, Robert W. Dickey, H. Ray Granade, Renuka Persad, and John S. Ramsdell

ABSTRACT

Forty great barracuda (*Sphyraena barracuda*) were caught between September 2001 and January 2002 in Marathon Key (Florida, USA) and analyzed for ciguatoxin activity. Two specific complementary methods were used for this purpose: the sodium channel site-5 receptor binding assay, and the ouabain veratridine-dependent cytotoxicity assay. A Caribbean ciguatoxin (C-CTX-1) was tested in both assays and compared to the dose-response of the brevetoxin congeners PbTx-1 or PbTx-3 to determine their relative potency and investigate the possibility of using brevetoxins as an internal standard. Sigmoidal calibration curves with Hill coefficients of 1 were obtained for both toxin classes in the two assays. Our results demonstrated that C-CTX-1 has an 8-fold higher affinity for the sodium channel than brevetoxins and a 440-fold higher potency in the cytotoxicity assay. Analysis of fish extracts calibrated against brevetoxin standard curves showed a linear relationship ($r^2=0.77$, slope 1.056 ± 0.2) between the two detection methods. Among the forty barracuda tested, 60% contained ciguatoxin levels that were above the detection limit of the cytotoxicity assay (0.039 ppb C-CTX-1 equivalents), and 30% were above 0.25 ppb, a level potentially harmful to humans. The most toxic fish contained 2.1 ppb C-CTX-1 equivalents. No correlation between barracuda size and toxin concentration was observed.



IN PRESS:

**** NEW!!! CHARACTERIZATION OF THE DEVELOPMENTAL TOXICITY OF CARIBBEAN CIGUATOXINS IN FINFISH EMBRYOS (CL# 1124, Toxicon)** ... Jamie R. Colman, Marie-Yasmine Bottein Dechraoui, Robert W. Dickey, and John S. Ramsdell

ABSTRACT

Since oviparous fishes mobilize fat stores to produce eggs, we investigated the potential for deposition of gonadal ciguatoxins to the oil laden yolk sacs which nourish developing embryos, and characterized the effects of these toxins on finfish development. Results showed that ciguatoxins are more concentrated in the egg mass (0.18 ng/g) of a toxic fish than in the muscle (<0.04 ng/g). We used a microinjection technique in a Japanese medaka (*Oryzias latipes*) developmental fish model to mimic the maternal route of toxin exposure to finfish embryos. We describe the developmental effects of two preparations isolated from Caribbean great barracuda (*Sphyraena barracuda*): a highly purified toxin (C-CTX-1), and ciguatoxins extracted from the flesh of a toxic fish. C-CTX-1 induced a significant decrease in heart rate after four days, which did not persist with further development. Crude extracts from ciguatoxic fish flesh induced hyperkinetic twitching and severe spinal deformities. These effects were observed in embryos receiving as little as 5 pg/egg, and were consistently found in embryos receiving doses exceeding 10 pg/egg. The occurrence of twitching and spinal deformities increased in both frequency and severity

with dose. Larvae suffering from spinal abnormalities were unable to orient themselves, and could not feed, resulting in mortality. The greater distribution of toxin to eggs as compared to flesh suggests that fish with low to moderate (0.5 ppb) flesh toxin levels would maternally transfer detrimental amounts of ciguatoxins to their offspring.

**** DEVELOPMENT AND APPLICATION OF LSU rRNA PROBES FOR *Karenia brevis* IN THE GULF OF MEXICO, USA (CL# 1109, Harmful Algae) ...** Tina Mikulski, Steve Morton and Greg Doucette

ABSTRACT

The brevetoxin producing dinoflagellate, *Karenia brevis*, is the target of several monitoring and research programs in the Gulf of Mexico where it forms extensive and frequently long-lived annual blooms that can cause severe economic losses. Rapid, reliable methods for the detection and enumeration of *K. brevis* cells, as well as their discrimination from morphologically similar species, are valuable tools for managers and scientists alike. Our aim was to produce a species-specific molecular probe that would serve as a tool to facilitate the efficient and reliable detection of *K. brevis* in the Gulf of Mexico. We sequenced a fragment of the large-subunit ribosomal RNA gene (LSU rDNA) from five *Karenia brevis* cultures isolated from the Texas Gulf coast, the Florida Gulf coast, and the Atlantic coast of Florida, and detected no differences among these isolates. A consensus sequence was thus compiled and compared to a previously published sequence from *K. mikimotoi*, the closest known phylogenetic relative to *K. brevis*, for the purpose of identifying unique *K. brevis* signature sequences. Fluorescently-labeled (FITC) oligonucleotide probes targeting these regions of the *K. brevis* LSU rRNA were designed to include at least two base pair differences, as compared to *K. mikimotoi*. Among seven probes designed, one uniquely identified all *K. brevis* isolates to the exclusion of all other species tested (Kbprobe-7), including a Gulf of Mexico *K. mikimotoi* isolate (Sarasota, FL) and several additional *Gymnodinium* species, as well as other dinoflagellate, diatom, and raphidophyte taxa. Importantly, *K. brevis* cells in samples taken during a 2001 bloom, fixed with a mixture of modified saline ethanol and 10% formalin, and stored at 4°C for seven months were successfully labeled with Kbprobe-7. In addition, preliminary analysis of labeled cells by flow cytometry revealed that *K. brevis* could be distinguished from *K. mikimotoi* in solution, suggesting other potential applications of this probe.

**** EXPRESSION OF A α,β,γ TUBULIN, THE MINIMAL SET OF TUBULINS REQUIRED TO DEFINE MICROTUBULE FUNCTION IN EUKARYOTIC CELLS, IN THE UNICELLULAR DINOFLAGELLATE, *Karenia brevis*. (CL# 1045, Phycologia) ...** Michèle Barbier et al. (Jeanine Miller, Steve Morton and Fran VanDolah)

ABSTRACT

Tubulin is a highly conserved family of proteins that are a major component of the microtubule cytoskeleton of eukaryotic cells. Here we report the presence of the three essential members of this family, α -, β - and γ -tubulin, in the unicellular dinoflagellate *Karenia brevis* by western blotting and immunolocalization. The cortical cytoskeleton and the intracytoplasmic structures are detailed by immunocytofluorescence techniques using antibodies to each tubulin on whole-permeabilized cells from laboratory cultures or field samples. The cortical microtubules could be visualized with anti- α - and anti- β -tubulin labeling revealing a morphology typical of dinoflagellates, while γ -tubulin was detected near the nucleus, probably associated with the archoplasmic sphere. The mitotic spindle, which arises from this region is described during different stages of mitosis. The cortical cytoskeleton does not depolymerize during mitosis, a feature that appears to be unique to dinoflagellates. For the first time, a detailed description of the cytoskeleton and the mitotic process is presented in the dinoflagellate *K. brevis*.



PUBLISHED:

**** THE TYPE B BREVETOXIN (PbTx-3) ADVERSELY AFFECTS DEVELOPMENT, CARDIOVASCULAR FUNCTION AND SURVIVAL IN MEDAKA (*Oryzias latipes*) EMBRYOS.** (CL# 1099, *Environmental Health Perspectives* 111(16): 1920-1925) ... Jamie Colman and John Ramsdell

ABSTRACT

Brevetoxins are produced by the Florida red tide dinoflagellate, *Karenia brevis*. The toxins are lipophilic polyether toxins, which elicit a myriad of effects depending upon the route of exposure. Brevetoxins are therefore broadly toxic to marine and estuarine animals. By mimicking the maternal route of exposure to the oocytes in finfish, we have characterized the adverse effects of the type 1 brevetoxin, brevetoxin (PbTx-3), on embryonic fish development and survival. The Japanese rice fish, Medaka (*Oryzias latipes*) was used as the experimental model in which individual eggs were exposed via microinjection to known concentrations of PbTx-3 dissolved in natural fish oil. Embryos injected with doses exceeding 1ng/egg displayed tachycardia, hyperkinetic twitches in the form of sustained convulsions, clumping of the erythrocytes, and decreased hatching success. Furthermore, fish dosed with toxin were often unable to hatch in the classic tail first fashion and emerged head first, which resulted in partial hatches and death. An LD₅₀ was determined wherein a dose of 4.0ng was sufficient to kill 50% of the fish, resulting in death. The results of this study complement previous studies of the developmental toxicity of the type 2 brevetoxin, brevetoxin-1 (PbTx-1). Furthermore, they provide information on the most commonly occurring brevetoxin and will illustrate *in vivo* the differing toxicities between the type 1 and type 2 brevetoxins.

**** PERMANENT EXPRESSION OF A CYCLIN B HOMOLOGUE IN THE CELL CYCLE OF THE DINOFLAGELLATE, *Karenia brevis* ...** (CL# 1037, *Journal of Eukaryotic Microbiology* 50(2): 123-31) Michèle Barbier, Tod Leighfield, Soyer-Gobillard, M.O. and Fran VanDolah

ABSTRACT

The eukaryotic cell cycle is driven by a set of cyclin dependent kinases associated with their regulatory partners the cyclins, which confer activity, substrate specificity and proper localization of the kinase activity. We describe the cell cycle of *Karenia brevis* and provide evidence for the presence of a cyclin B homologue in this primitive eukaryotic dinoflagellate. This cyclin B homologue has an unusual behavior, since its expression is permanent and its localization is cytoplasmic throughout the cell cycle. This behavior is similar to a cyclin B homologue, p56, previously described in a different species of dinoflagellate. However, in *K. brevis*, the cyclin B homologue is also present in the nucleus, specifically bound to the nucleolus during interphase. There is no evidence for the translocation to the nucleus during mitosis. Here we discuss the unique behavior of the cyclin B homologue in dinoflagellates, its relationship to the unusual characteristics of dinomitosis, and its potential implications regarding the evolution of cell cycle regulation among eukaryotes.

**** MEASUREMENT OF BREVETOXIN LEVELS BY RADIOIMMUNOASSAY OF BLOOD COLLECTION CARDS AFTER ACUTE, LONG-TERM AND LOW DOSE**

EXPOSURE IN MICE. (CL# 1080, *Environmental Health Perspectives* 111(13): 1595-1600) ...

Ricky Woofter, M-Yasmine Bottein Dechraoui, Ian Garthwaite, Neil R. Towers, Christopher J. Gordon, José Córdova and John S. Ramsdell

ABSTRACT

A radioimmunoassay (RIA) has been developed using a sheep anti-brevetoxin to evaluate detection of brevetoxin on blood collection cards from mice treated with the brevetoxin congener (PbTx-3). The RIA was designed in similar format to receptor assay to facilitate comparison with previous work with blood collection cards. The RIA uses a 1/4000 dilution of sheep antiserum, 0.4 nM [³H]-PbTx-3, and goat antisheep IgG-cellulose with separation on glass fiber filters. The receptor binding assay (RBA), using rat brain membrane, has an affinity for PbTx-3 ($EC_{50} = 4.3 \pm 1.5$ nM, $n=7$) and recognizes type 1 and type 2 brevetoxins, as well as ciguatoxin. Whereas the RIA, using a PbTx-2 specific antibody, has an affinity for PbTx-3 ($EC_{50} = 1.2 \pm 0.2$ nM, $n=10$) and recognizes both type 1 and type 2 brevetoxins, but not ciguatoxin. Comparison of the different brevetoxin subtypes affinity using RIA and RBA yields a rank order of potency where $PbTx\ 6 > 3 = 2 = 9 > 1$. Thus, the two assays provide comparable values for the commonly occurring PbTx-2 and 3 as well as PbTx-9, while showing differences for PbTx-6 and PbTx-1. We next compared the two assays by measuring brevetoxin in the blood of mice exposed to a sublethal dose, 180 µg/kg of PbTx-3 for 0.5, 1, 2, 4, and 24 hr. The blood from each mouse was preserved on blood collection cards. Each 0.1 ml blood spot was extracted in 2 ml methanol. This extract was then tested by both assays. The RBA reported the blood brevetoxin activity (at 2 hr brevetoxin activity was detected in 3 of 4 mice), while the RIA gave blood brevetoxin levels (at 2 hr: 25.75, 28.27, 39.26, 28.51 nM PbTx-3). Taken together these results show the value of tier-based testing for brevetoxin: antibody methods provide a good screening method that may detect metabolites; receptor-based methods provide a good toxicity measurement and LC-MS/MS provides absolute confirmation of toxin congeners.

**** RE-EVALUATION OF PARALYTIC SHELLFISH TOXIN PRODUCTION BY BACTERIA ASSOCIATED WITH DINOFLAGELLATES OF THE PORTUGUESE COAST. ...** (CL# 1085, *Applied and Environmental Microbiology* 69: 5693-5698) Martins, C.A., Alvito, P., Tavares, M.J., Pereira, P., Doucette, G.J. and S. Franca

ABSTRACT

Paralytic Shellfish Toxins (PSTs) are a suite of potent neurotoxins whose production is associated with certain dinoflagellate and cyanobacterial species. The autonomous production of PSTs by some bacterial strains, namely those associated with PST producing dinoflagellates, remains controversial. In addition to reports on their PST production, there is some evidence to suggest that certain compounds in some bacterial isolates were incorrectly identified as PSTs by HPLC analysis. In the current study, PST production by two bacterial strains, *Pseudomonas stutzeri* and *Pseudomonas diminuta*, isolated from *Alexandrium lusitanicum* and *Gymnodinium catenatum*, respectively, was evaluated using a mouse neuroblastoma (MNB) assay and the results compared to HPLC analyses of the same samples. Since we have previously assessed the presence of PSTs in these bacterial isolates by HPLC, results of the present study are also discussed in relation to our earlier findings. Toxicity studies were performed under optimal conditions for toxin production and detection, as described in the published literature. PSTs were not detected by HPLC analysis in either supernatants or bacterial cell extracts. Analysis by MNB assay was negative for supernatants but initially positive for crude extracts. Nonetheless, this positive assay response was eliminated following C18 sep-pak clean-up of the extracts, indicative of a matrix effect on the assay and thus the absence of PSTs in these samples. We conclude that neither our MNB nor HPLC data are consistent with autonomous bacterial PST production under the study conditions.

**** IN VITRO ASSAYS FOR PHYCOTOXINS...** (CL# 1038, In: Hallegraeff, G.M., Anderson, D.M. & Cembella, A.D. (eds.), *Manual on Harmful Marine Microalgae*. Second Edition. Monographs on Oceanographic Methodology, 11. IOC-UNESCO, Paris. pp. 297-345.) ...A.D. Cembella, Greg Doucette and Ian Garthwaite

ABSTRACT

Not available in electronic form at this time (see Greg)

**** A RECEPTOR BINDING ASSAY FOR PARALYTIC SHELLFISH POISONING TOXINS: OPTIMIZATION AND INTERLABORATORY COMPARISON ... (CL# 1025, J. Assoc. Offic. Anal. Chem. 86: 737-745)** Ruberu, S.R., Liu, Y.G., Wong, C.T., Perera, S.K., Langlois, G.W., Doucette, G.J. and C.L. Powell

ABSTRACT

A receptor binding assay (RBA) for detection of paralytic shellfish poisoning toxins was formatted for use in a high throughput detection system employing microplate scintillation counting. The RBA technology was transferred from the National Ocean Service (NOS), which uses a Wallac TriLux 1450 MicroBeta microplate scintillation counter, to the California Department of Health Services (CDHS), which uses a Packard TopCount instrument. Due to differences in the detector arrangement between these two counters, markedly different counting efficiencies were exhibited, requiring optimization of the RBA protocol for the TopCount instrument. Precision, accuracy, and sensitivity (LOD = 0.2 mg STX equiv./100 g shellfish tissue) of the modified protocol were equivalent to those of the original protocol. The RBA robustness and adaptability were demonstrated by an interlaboratory study, in which STX concentrations in shellfish generated by the TopCount were consistent with MicroBeta-derived values. Comparison of saxitoxin reference standards obtained from the FDA and the National Research Council, Canada showed no observable differences. This study confirms the RBA's value as a rapid, high throughput screen prior to testing by the conventional mouse bioassay (MBA) and suitable for providing an early warning of increasing PSP toxicity when toxin levels are below the MBA limit of detection.

**** CULTURE METHODS (CL# 1001, In: Hallegraeff, G.M., Anderson, D.M. & Cembella, A.D. (eds.), Manual of Harmful Algae, pp.77-98)...** R. R. L. Guillard and S.L. Morton

ABSTRACT

Not available in electronic form at this time. See Steve Morton.

**** LEARNING IMPAIRMENT CAUSED BY INFUSION OF A TOXIN PRODUCED BY *Pfiesteria piscicida* INTO THE HIPPOCAMPUS OF RATS (CL# 1079, Neurotoxicology and Teratology 25: 419-426)...** Edward D. Levin et al. (Peter D. R. Moeller and John S. Ramsdell)

ABSTRACT

Pfiesteria piscicida, an estuarine dinoflagellate, which has been shown to kill fish, has also been associated with neurocognitive deficits in humans. With a rat model, we have demonstrated the cause-and-effect relationship between *Pfiesteria* exposure and learning impairment. In several studies, we have replicated the finding in Sprague-Dawley rats that exposure to fixed acute doses of *Pfiesteria* cells or filtrates caused radial-arm maze learning impairment. Recently, this finding of *Pfiesteria*-induced learning impairment in rats has been independently replicated in another laboratory as well. We have demonstrated significant *Pfiesteria*-induced learning impairment in both the win-shift and repeated acquisition tasks in the radial-arm maze and in reversal learning in a visual operant signal detection task. These learning impairments have been seen as long as 10 weeks after a single acute exposure to *Pfiesteria*. In the current study, we used a hydrophilic toxin isolated from clonal *Pfiesteria piscicida* cultures (PfTx) and tested its effect when applied locally to the ventral hippocampus on repeated acquisition of rats in the radial-arm maze. Toxin exposure impaired choice accuracy in the radial-arm maze repeated acquisition procedure. The PfTx-induced impairment was seen at the beginning of the session and the early learning deficit was persistent across six weeks of testing after a single administration of the toxin. Eventually with enough practice each session the PfTx exposed rats did learn that session's problem as did control rats. This model has demonstrated

the cause-and-effect relationship between exposure to a hydrophilic toxin produced by *P. piscicida* and learning impairment and specifically that the ventral hippocampus was critically involved.